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The effect of salinity and temperature on the uptake of cadmium and zinc by the common blue mussel, *Mytilus edulis* with some notes on their survival

M. Ali[1] and A. Taylor[2*]
[1]*Aquaculture, Fisheries and Marine Environment Department, Kuwait Institute for Scientific Research, P.O. Box 1638, Salmiya 22017, Kuwait*
[2]*Faculty of Biomedical & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, United Kingdom*

**e-mail: a.taylor@bio.gla.ac.uk*

Abstract - This study has investigated the effect of salinity and temperature on the rates of uptake of Cd and Zn by the blue mussel *Mytilus edulis* under laboratory conditions. Exposure of mussels to concentrations of 2 ppm resulted in an almost linear increase in the concentration of Cd and Zn throughout 14 days of exposure. Only the whole soft tissue of each mussel was frozen, freeze-dried and acid-digested before measuring the level of contamination by the spectrophotometer. Zn uptake was interrupted by elimination periods at high salinity. The uptake of Cd by *M. edulis* was at its maximum at low salinity and high temperature while at high salinity, temperature did not significantly affect the rate of uptake. Salinity had a significant effect on Zn uptake with the rate of uptake being higher at low salinity. Mortalities of mussels exposed to Cd and Zn along with controls were only observed at low salinity.

Keywords: *Mytilus edulis*, zinc, cadmium, temperature, salinity.

Introduction

Mussels, such as *Mytilus edulis,* are common in and around estuaries worldwide and are an important food source for humans both in local populations (Farrington *et al*., 1983; Gold-Bouchot *et al.,* 1993; Jaffe *et al.,* 1995; Kyle *et al*., 1997) and commercially (Farrington *et al*., 1983; Bussell *et al*., 2008). The international catch of marine molluscs in 2004 was 2.05 million tons, of which 50% consisted of *M. edulis,* representing 6% of all aquaculture production (FAO, 2006). In addition, mussels also provide an important habitat for other invertebrates and algae (Seed, 1996) increasing local biodiversity as well as performing a range of other important ecosystem functions in their role as filter feeders (Ostroumov, 2005), by increasing primary productivity (Pfister, 2007) and nutrient cycling (Prins and Smaal, 1994). The common mussel, *M. edulis*, is also considered to be an important item in the food web since they are preyed upon by many species including the red king crab *Paralithodes camtschaticus* (Sokolov and Milyutin, 2006), and the asteroid starfish *Asterias vulgaris* (O'Neill *et al*., 1983).

M. edulis has become a popular organism to study heavy metal accumulation especially through biomonitoring programmes due to their longevity, sessile nature, filter feeding activity, ability to accumulate toxins and their global distribution (Farrington *et al*., 1983; Goldberg, 1986; Widdows and Donkin, 1992; Beliaeff *et al*., 1997; Miller, 1999). Moreover, *M. edulis* is a keystone species, and heavy metals or other pollutants may have an impact on the immune system and other aspects of the physiology of this species which, in turn, will affect the health of mussel beds within or near estuaries, which could have significant effects on the broader ecosystem (Bussell *et al*., 2008). These mussels along with other marine biota are exposed to different types of contaminants mainly introduced to the sea by man and the most important forms of these pollutants are hydrocarbons (e.g. Neff *et al*., 2006) and heavy (trace) metals.

The aim of this study was to examine some of the factors that affect heavy metal accumulation in the mussel *M. edulis*. This species was chosen because bivalves and gastropods are very suitable as *in situ* monitors of heavy metal accumulation since they are abundant, large, sedentary and easy to collect (Hartley and Johnston, 1983). Metal concentration in molluscs depends on a number of factors such as the concentration of the element in the environment, the chemical species and their interaction with other pollutants but also on the animals' size, age, growth rate, sex, reproductive state, gut content, reproductive season of individuals and also on environmental factors such as salinity, temperature and pH (Phillips, 1976a,b; 1977b; 1980; Watling and Watling, 1976; Boyden, 1977; Davenport, 1977; Cossa *et al*., 1979; 1980; Lobel and Wright, 1982; Lobel *et al*., 1991; Brown and Luoma, 1995; Langston and Spence, 1995; Stecko and Bendell-Young, 2000; Conti, 2002).

Metal accumulation in the tissues of bivalves and gastropods is proportional to the degree of contamination in the environment and can be used as an indicator of metal pollution in the marine environment (Goldberg *et al*., 1983; Elder and Mattraw, 1984). Furthermore, environmental parameters such as temperature and salinity are very influential on the rate of uptake and accumulation of different trace metals by marine organisms including *M. edulis* (e.g. Phillips, 1977b; Mubiana and Blust, 2007). For instance, it has been reported that Cd uptake by *M. edulis* and other molluscs is affected by salinity (Jackim *et al*., 1977). Similarly, the net uptake of Zn from the surrounding seawater is elevated by the presence of highly stressful salinity states, for example, a rapid increase in salinity following a period of decreasing salinity (Phillips, 1977b). Salinity is potentially a major environmental driver of mussel health (Bussell *et al*., 2008). Reduced salinity is known to affect several metabolic and physiological parameters in mussels including heart rate (Bakhmet *et al*., 2005; Braby and Somero, 2006), respiration rate (Stickle and Sabourin, 1979), energy obtainment (Gardner and Thompson, 2001) and growth rate (Westerbom *et al*., 2002) leading to potentially important impacts for the health of the animal (Oliver, 2007). Increased salinity has been shown to hinder haemocyte circulation and locomotion (Fisher, 1988) while decreased salinity results in greater haemocyte mortality (Gagnaire *et al*., 2006).

The general assumption is that metal uptake is directly proportional to temperature within the normal metabolic range (Jackim *et al*., 1977) but this hypothesis is sometimes incorrect (e.g. Fowler and Benayoun, 1974). For example, at low temperature, copper (Cu) concentration increased in the tissues, but there was a sudden decrease with a change to high temperature (Myint and Tyler, 1982) whereas the uptake of Cd (Jackim *et al*., 1977; Lannig *et al*., 2006) and Zn (Baines *et al*., 2006) were directly proportional to temperature.

Environmental stresses rarely occur in isolation and stress from natural and anthropogenic physical disturbance is also known to affect the immune function of bivalves (Bussell *et al*., 2008). Therefore, such weakness due to stressful conditions caused by extreme salinity and temperature will affect the health of the mussels and therefore make them easily susceptible to diseases (Bussell *et al*., 2008) and possibly to heavy metal accumulation.

In the present study, the effect of temperature and salinity on the uptake and toxicity of Cd and Zn in the mussel *M. edulis* was examined. In this experimental study, the null hypothesis tested was that neither salinity nor temperature has any direct affect on the uptake of heavy metals by *M. edulis.*

Materials and Methods:

Collection of mussels

Samples of the common mussel *M. edulis* were collected manually during low-tide from Lunderston Bay, south of Gourock, Firth of Clyde, Scotland during April-May 2008. The mussels were selected to be of approximately similar size (shell length = 26-57.7 mm; fresh weight = 0.39-7.88 g). The mussels were then transferred to a recirculating seawater aquarium (temperature = 6±2°C; salinity = 35±3 PSU) at the University of Glasgow and placed in a tank provided with continuous aeration. The mussels were subjected to a 12:12 hour light:dark cycle. The mussels were not fed during the experiments but were able to feed on particulate matter in the recirculating seawater.

Preliminary experiments:

a) Preliminary experiment-1:

A preliminary experiment was carried out to test the accuracy and repeatability of the analytical procedure adopted for this study. Tissue from five 'control' mussels was combined to provide sufficient material to provide seven replicate samples. After combining the tissue samples, the material was mixed very thoroughly by grinding using a pestle and mortar. Seven replicate samples (100 mg each) were weighed out from this tissue sample and digested in nitric acid as described below. Following digestion and centrifugation, the concentrations of Cd and Zn in the seven samples were determined by atomic absorption spectrophotometry.

b) Preliminary experiment-2:

A second preliminary experiment was carried out to determine the accuracy of the experimental procedures used in the analysis. Samples of lobster hepatopancreas tissue was used as reference material. Careful analysis of this tissue had been carried out by the laboratories of the National Research

Council of Canada (NRCC) to determine the concentrations of a range of heavy metals. The NRCC has certified the accuracy of the concentrations of different heavy metals within this tissue. Five samples (100 mg each) of the lobster hepatopancreas tissue were digested and then the concentrations of Cd and Zn were determined using the procedures described above.

Experiment 1:

This experiment was carried out to study the combined effect of salinity and temperature on the rate of uptake of Cd and Zn. Control tanks (volume =2 l) containing filtered seawater were placed in constant temperature rooms at 6±2°C and 12±2°C along with 6 tanks (3 at each temperature) containing 2 l of filtered seawater (salinity = 35±3 PSU in all tanks) having a concentration of the trace metal being tested of 2.00 ppm. For all 3 metals, this concentration was achieved by adding the appropriate amount of the nitrate salt of the metal. All tanks had continuous aeration. Each tank contained 20 mussels of approximately the same size and were exposed to one of the trace metals in order to test the rate of uptake of each at the different temperatures. The experiment lasted for 14 days. The water was changed every 3 days and simultaneously, samples of 30 mussels (5 from each temperature) were chosen randomly for metal analysis. Changing the water in the tanks every 3 days ensured that the metal concentration remained at 2.00 ppm throughout the experiment. As for controls, samples of 10 mussels (5 from each temperature) were taken on day 0, day 7 and day 14 from animals kept in tanks under the same conditions but in seawater without Cd or Zn being added to the water. The temperature of the water in the tanks was checked regularly using a digital thermometer, while salinity was measured using a refractometer (Atago, Japan).

Experiment 2:

This experiment was also carried out to study the combined effect of salinity and temperature on the rate of uptake of Cd and Zn. Salinity was adjusted to 20±3 PSU in all tanks by dilution of the 'stock' seawater using fresh water. Control tanks (volume = 2 l) containing filtered seawater were placed in constant temperature rooms at 6±2°C and 12±2°C together with 6 tanks (3 at each temperature) containing 2 l of filtered seawater. All tanks (except controls) had a metal concentration of 2.00 ppm. Each tank contained 20 mussels exposed to one of the trace metals. The experiment lasted for 14 days. As in the previous experiment, the water was changed every 3 days and simultaneously, samples of 30 mussels (5 from each temperature) were randomly chosen for metal analysis. Controls (10 mussels, 5 from each temperature) were taken at day 0, day 7 and day 14.

Sample preparation:

a) Dissection:

After removal from the tanks, the lengths of the mussels (anterior:posterior axis) were measured using dial Vernier calipers. They were then dissected in the laboratory using forceps and a scalpel, which were rinsed with distilled water after each dissection in order to avoid contamination. The wet weight of the total soft tissue from each mussel was determined using a four decimal place balance and then transferred to

labeled glass vials and kept in a freezer (-20°C) for 24-48 hours. Afterwards, the samples were placed in a freeze-dryer (Edwards Modulo) (-40°C) for 48-72 hours until dry.

b) Digestion:

After freeze-drying, the tissue samples were ground to a homogeneous powder using a ceramic pestle and mortar. 100 mg of each sample was transferred into 50 ml conical flasks. A calibrated pump was used to add 10 ml of concentrated nitric acid to the 50 ml conical flasks under a fume hood. The flasks were then placed over a hot plate and simmered until digestion was complete (i.e. when the sample was clear and orange/brown fumes were no longer visible). This usually took between 60-90 minutes. The digested samples were left to cool at room temperature and then each sample was carefully poured into a 10 ml volumetric flask. The sides of the 50 ml flask were rinsed with distilled water using a disposable Pasteur pipette and the volume of liquid in the volumetric flask made up to the 10 ml mark with distilled water.

Heavy metal analysis:

Samples were analyzed for Cd and Zn using a Philips 9200 flame atomic absorption spectrophotometer, fitted with the correct lamp and using the appropriate wavelength as specified for each metal. Calibration of the spectrophotometer was performed using standard solutions of a suitable concentration range: Cd (0.500 ppm, 0.250 ppm, 0.125 ppm) and Zn (0.500 ppm, 1.00 ppm, 2.00 ppm). Samples were analyzed using an air-acetylene flame with distilled water aspiration between each analyzed sample. The aspiration tube was wiped with tissue after each sample then returned to distilled water in between each analysis.

Statistics:

Parametric statistical analyses using the general linear model (GLM) were performed by Minitab software version 15.0. GLM was chosen because it provides a synthetic understanding of a large range of common statistical methods that includes Regression and classical Analysis of Variance (ANOVA).A p value of <0.05 was used to indicate that the relationship tested was significant.

Results:

Preliminary experiments:

a) The results from the preliminary experiment that was carried out to test the accuracy and repeatability of the analytical procedure adopted for this study are presented in Table 1. The data show that the repeatability of the measurements was high with low values for the coefficient of variance. The exception was for the Cd data where the coefficient of variance was higher but this is probably attributable to the very low concentrations of Cd that were present in the tissues of control mussels. The high repeatability or precision of the values determined for each metal gave confidence in the procedures used to prepare and analyze the samples.

Table 1. The concentration (μg.g-¹) of each metal from the seven replicate samples. The values shown are the individual measurements of each of the seven samples together with the overall mean ± SD. Values for the coefficient of variance ("SD/mean"x100) are also shown.

Sample	Cd	Zn
1	0.4	364
2	0.4	332
3	0.2	344
4	0.2	396
5	0.2	342
6	0.2	386
7	0.21	358
Mean±SD	0.36±0.097	360.27±23.65
Coefficient of Variance	26.8%	66%

b) A second preliminary experiment was carried out to determine the accuracy of the experimental procedures used in the analysis. This was done by using exactly the same procedures used in the rest of this study to determine the concentrations of Cd and Zn in samples of lobster hepatopancreas tissue supplied by the National Research Council of Canada (NRCC). The values obtained were compared with those obtained by the NRCC which has certified the accuracy of the concentrations of different heavy metals within this tissue. The results obtained from the analysis of the 5 replicate samples of this tissue are shown in Table 2.

Table 2. The concentrations (μg.g-¹) of each metal from the five replicate samples of the lobster hepatopancreas tissue. The values shown are the means ± SD. The certified concentrations of each of the metals published by the NRCC for the same tissue are also shown as means ± SD.

	Cd	Zn
This study	33.14±3.14	178.5±14.79
NRCC values	26.7±0.60	180±6

The data obtained from the analyses of this tissue compare very closely with the values provided by the NRCC and indicate that the analytical procedures used in this study provide an accurate measure of the concentrations of heavy metals in the tissues of the mussels.

Survival of Mytilus edulis exposed to cadmium and zinc:

The survival rates of mussels exposed to 2 ppm concentrations of each of the metals are shown in Table 3. Mussels exposed to Zn had the highest survival rates followed by those exposed to Cd. It was noticeable that in all cases, the survival was lowest when the mussels were exposed to the metals at low salinity. Temperature, however, did not appear to have a major impact on survival. Furthermore, mortalities of controls were only encountered at low salinity.

Table 3. Survival percentage (%) of *Mytilus edulis* exposed to Cd and Zn along with control mussels within the 14-day exposure period under 35±3 PSU and 20±2 PSU at 6±2 °C and 12±2°C.

Condition	Cd	Zn	Controls
35 PSU, 6°C	100	100	100
35 PSU, 12°C	100	100	100
20 PSU, 6°C	64	60	44
20 PSU, 12°C	64	92	68

Cadmium accumulation by Mytilus edulis:

The accumulation of Cd by mussels exposed to different combinations of temperature and salinity is shown in Figure 1. In all cases, the concentration of Cd in the control mussels remained low (<1.41µg.g-1) throughout the experiment. There was also very little variation between the control mussels in the measured Cd concentration resulting in the SD values being too small to be visible in Figure 1.

a) *Salinity-temperature interactions in the presence of cadmium:*

The interaction between temperature and salinity was tested statistically and it was found to be significant ($F_{1,64}$; $p=0.040$) and it was very obvious at low salinity and high temperature as will be explained below.

b) *Cadmium uptake at two temperatures under conditions of high salinity:*

The results of this experiment showed that the mussels readily took up Cd during the 14-day exposure period. There was a gradual increase in the accumulation of Cd throughout the 14-day exposure period under both temperature regimes at high salinity (35 PSU). It was found that the mean Cd concentrations on days 3, 7, 11 and 14 were slightly higher at the higher temperature (12°C) than those at lower temperature (6°C) (Fig.1). The rate of uptake was approximately constant during this period as indicated by the regression lines (Fig.1).

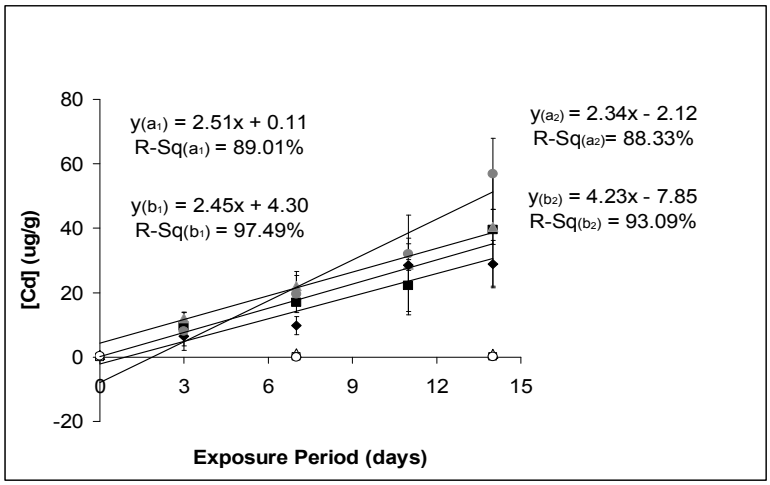
The rate of uptake of Cd by mussels maintained in seawater having a salinity of 35 PSU did not differ significantly between those mussels held at 6°C and 12°C (Fig.1) since the rate of uptake of Cd at 6°C was 2.51 µg.g-1.day-1, while at 12°C it was 2.45 µg.g-1.day-1 (Fig.1), which shows that changes in temperature of this magnitude did not have a significant effect on the Cd uptake in mussels kept at 35 PSU (Fig.1). Therefore the interaction between salinity and temperature ($F_{1,64}$; $p=0.040$) at high salinity is not so obvious.

c) *Cadmium uptake at two temperatures under conditions of low salinity:*

The results from this experiment showed that there was a significant effect of temperature on the rate of uptake of Cd by mussels kept at low salinity (20 PSU) (Fig.1). The rate of uptake of Cd was significantly higher at the higher temperature (12°C) than at the lower temperature (6°C) ($F_{1,64}$; $p=0.040$) (Fig.1). There was a gradual and continuous increase in the accumulation of Cd throughout the 14-day exposure period at 12°C but it is

clear that the mean Cd concentrations on days 3, 7, 11 and 14 are significantly higher (almost double) the values found in mussels kept at 6°C. The calculated rate of uptake at 6°C was 2.34 µg.g-¹.day-¹, while at 12°C it was 4.23 µg.g-¹.day-¹ (Fig.1), which shows that the Cd uptake by mussels kept at low salinity is strongly controlled by temperature and salinity itself has a weak effect on the rate of Cd uptake especially at low temperature (Fig.1).

Figure 1. The uptake of Cd by *Mytilus edulis* exposed to a Cd concentration of 2 ppm over a 14-day exposure period (closed symbols). Values for control mussels (open symbols) kept under the same conditions but not exposed to Cd are also shown. The mussels were kept in seawater having a salinity of 20±3 PSU at temperatures of 6±2 °C (black diamonds) and 12±2 °C (grey circles) and 35±3 PSU at temperatures of 6°C (black squares) and 12°C (grey triangles). Regression equations and R-Sq values are shown: (a1) at 6°C and (b1) at 12°C at 35 PSU and: (a2) at 6°C and (b2) at 12°C at 20 PSU. Values are means ± SD, n = 5.



Zinc accumulation by Mytilus edulis:

The accumulation of Zn by mussels exposed to different combinations of temperature and salinity is shown in Figure 2. In all cases, the concentration of Zn in the control mussels remained relatively low (<537µg.g-¹) throughout the experiment. There was also very little variation between the control mussels in the measured Zn concentration resulting in the SD values being too small to be visible for most of the controls on Fig. 2.

a) *Salinity-temperature interactions in the presence of zinc:*

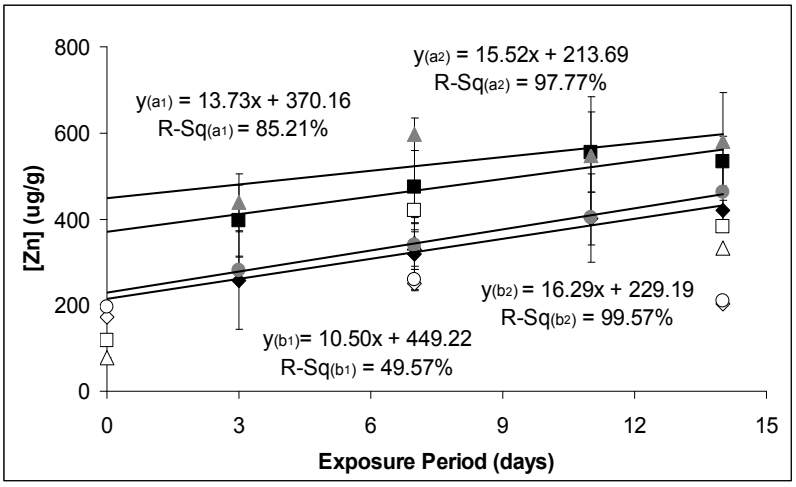
The interaction between temperature and salinity was also tested statistically but it was not significant ($F_{1,64}$; $p = 0.427$). The GLM also showed that the effect of temperature was marginally significant ($F_{1,64}$; $p = 0.045$) and this could be explained by the slight increase in the rate of uptake under the higher temperature at low salinity (Fig.2) but the opposite was the case at high salinity (Fig.2) where the effect of salinity was highly significant ($F_{1,64}$; $p < 0.001$). Furthermore, such differences in the rate of uptake between temperatures could also be related to elimination periods (Fig.2) as will be explained below.

b) *Zinc uptake at two temperatures under conditions of high salinity:*

There was a gradual increase in the accumulation of Zn throughout the 14-day exposure period under both temperature regimes in mussels maintained in a high salinity environment but the increase in the concentration of Zn in the tissues did not follow the same linear increase that was observed for Cd. Thus, there was a slight decrease in Zn uptake on day 14 in mussels kept at 6°C and another slight decrease was observed on day 11 at 12°C (Fig.2). The R-Sq value for the regression lines fitted to the data obtained at 6°C was high but the R-Sq value for the 12°C data was lower indicating that the regression equation may not provide the best description of the relationship between Zn uptake and time at this temperature (Fig.2). It is clear, however, that the mean Zn concentrations on days 3, 7, 11 and 14 were slightly higher in mussels kept at 12°C than in those kept at 6°C (Fig.2).

The results of this study have shown that the effect of temperature was marginally significant ($F_{1,64}$; $p=0.045$) on the rate of uptake of Zn by mussels at 35 PSU. The calculated rate of uptake at 6°C was 13.73 µg.g-¹.day-¹, while at 12°C it was 10.5 µg.g-¹.day-¹ (Fig.2), which shows that the Zn uptake in high salinity was strongly controlled by salinity ($F_{1,64}$; $p<0.001$) but temperature has no significant effect. Moreover, it seems that there is an inverse relationship between the rate of Zn uptake and salinity, since the rate of uptake was higher at a salinity of 20 PSU as indicated by the regression equations (Fig.2).

Fig. 2. The uptake of Zn by *Mytilus edulis* exposed to a Zn concentration of 2 ppm over a 14-day exposure period (closed symbols). Values for control mussels (open symbols) kept under the same conditions but not exposed to Zn are also shown. The mussels were kept in seawater having a salinity of 20±3 PSU at temperatures of 6±2 °C (black diamonds) and 12±2 °C (grey circles) and 35±3 PSU at temperatures of 6°C (black squares) and 12°C (grey triangles). Regression equations and R-Sq values are shown: (a1) at 6°C and (b1) at 12°C at 35 PSU and: (a2) at 6°C and (b2) at 12°C at 20 PSU. Values are means ± SD, n = 5.



c) *Zinc uptake at two temperatures under conditions of low salinity:*

As was observed in mussels kept at high salinity, there was a gradual increase in the accumulation of Zn throughout the 14-day exposure period under both temperature regimes. However, as with mussels kept in low salinity conditions, the rate of uptake was linear in comparison to Zn uptake at high salinity (Fig.2). The mean Zn concentrations on days 3, 7, 11 and 14 were slightly higher at 12°C than at 6°C (Fig.2) but the differences were not significant since the calculated rate of Zn uptake by mussels in 20 PSU water at 6°C was 15.52 µg.g-¹.day-¹, while at 12°C it was 16.29 µg.g-¹.day-¹ (Fig.2). Comparison of the regression lines showed that there was no significant difference between the lines indicating that the temperature did not have a significant effect on the rate of Zn ($F_{1,64}$; $p=0.045$) uptake in mussels kept at 20 PSU in comparison to salinity ($F_{1,64}$; $p<0.001$). The higher rate of uptake at low salinity in comparison to high salinity suggests an inverse relationship between the uptake of Zn and salinity (Fig.2).

Discussion:

There is a growing literature on the effects of environmental variables on the rates of uptake of heavy metals in molluscs. For example, Phillips (1976a) reported that Cd uptake was increased in *M. edulis* near to freshwater inputs of trace metals, which are areas of low salinity. The trace metal content of an organism might be affected by the surrounding salinity in three different ways: (1) Many metals tend to be readily available to the biota in low salinity areas due to the higher capacity of freshwater than marine water to sustain metals in the water column, either in suspension or in solution (Bryan and Hummerstone, 1973a; Phillips, 1976a), (2) Various stable salinities might cause different rates of trace metal uptake by biota (e.g. O'Hara, 1973a,b) due to either gross physiological changes in the organism (e.g. in drinking and the rates of water filtration) (Phillips, 1977b) or to a connection of ion fluxes across the organism's body surface (Wolfe and Coburn, 1970; Bryan and Hummerstone, 1973b), (3) Salinity fluctuations might be stressful to an organism and could result in physiological changes such as valve closure in osmoconforming bivalve molluscs (Gilles, 1972; Coleman, 1973).

The common mussel, *M. eduilis* is known to be fairly tolerant of low salinity. Under laboratory conditions, the lowest salinity *M. edulis* have survived was 3.4 PSU for 9 hours (Costa and Pritchard, 1978), but the lowest recorded salinity at which *M. edulis* can exist in the field was 15 PSU (e.g. Phillips, 1976a; Fischer, 1988). It was also reported that the maximum salinity was 18-20 PSU in the Baltic Sea (Struck *et al*., 1997). These populations have adapted to these conditions over generations. In the Clyde Sea area, *M. edulis* would normally be exposed to rather higher salinities but the salinity of 20 PSU chosen for the experiments in the current study should have been within the tolerance range for this species and should therefore not have caused extreme stress.

Temperature has also been reported to affect the rate of uptake of heavy metals by *M. edulis* (Jackim *et al*., 1977; Myint and Tyler, 1982; Lannig *et al*., 2006) but currently, according to Lannig *et al*. (2006), few studies are

available that have addressed the interactive effects of toxic metals and increased temperatures in aquatic poikilotherms (Denton and Burdon-Jones, 1981; Sokolova *et al*., 2004; Dunca *et al*., 2005; Hallare *et al*., 2005; Cherkasov *et al*., 2006; Lannig *et al*., 2006).

Phillips (1976a) has shown that in *M. edulis* maintained at a high salinity (35 PSU) and low temperature (10°C), the Zn concentration in the tissues on day 14 was slightly higher than that at the higher temperature (18°C), which contrasts with the present results (Table 4). The effects of such salinity regimes on the net uptake of Cd and Zn were consistent with the effects of stable salinities and the period of actual decrease of salinity in the presence of metals appears to have had a unique qualitative effect on the metal uptake rates (Phillips, 1977b).

Zn uptake was also reported to exhibit an inverse relationship with salinity in *M. edulis* (Phillips, 1977b). Table 4 presents data from this study and that of Phillips (1976a). As for Cd, it could enter aquatic organisms, at least in part, by the same uptake mechanism as calcium (Ca) via calcium channels (Wright and Frain, 1981) because both elements have almost equal ionic radii, thus the higher the Ca turnover the higher the Cd uptake, since competition between Ca and Cd for binding sites on calcium transporting proteins is indeed possible (Belcheva *et al*., 2006).

Philips (1976a) recorded high values of Zn, Cd and lead (Pb) in the intertidal mussels and this could be applied to the mussels in this study since they were collected from the upper intertidal zone of Lunderston Bay. Phillips (1977a) reported that Zn and Cd concentrations in *M. edulis* collected from low salinity waters were higher than in mussels from high salinity waters. In addition, no effect of either salinity or temperature on the net uptake of Zn by *M. edulis* within the following limits: 15 PSU and 35 PSU; 10°C and 18°C (Phillips, 1976a). In this study, temperature and salinity ranges (20 PSU and 35 PSU; 6°C and 12°C) are closer to the ones reported by Phillips (1976a), thus, the uptake of Zn appeared to be stable and continuous at the lower salinity. This was not the case at high salinity and high temperature, at which there was an increase in Zn accumulation from day 3 to day 7 followed by a slight decrease (48.40 $\mu g.g^{-1}$) on day 11 and then followed by a slight increase on day 14 (33.60 $\mu g.g^{-1}$), while at the lower temperature, the uptake increased continuously during the exposure period except that there was a slight decrease on day 14 (20.92 $\mu g.g^{-1}$) (Fig.2). A marginally significant ($F_{1,64}$; $p=0.045$) effect of temperature was observed but there was no interaction between salinity and temperature ($F_{1,64}$; $p=0.427$). This could explain the low rate of Zn uptake at 35 PSU at 12°C (10.5 $\mu g.g^{-1}.day^{-1}$), which is lower than that at 6°C at 35 PSU that was 13.73 $\mu g.g^{-1}.day^{-1}$ having the lowest R-Sq value (49.57 %) among the Zn and Cd experiments (Fig. 2). The reasons behind such events were not obvious but this could be due to periods during which elimination (Luten *et al*., 1986), valve closure (Coleman, 1973) or the equivalent rate of excretion of accumulated body Zn that matches the rate of uptake (White and Rainbow, 1984), which has been shown to occur in decapod crustaceans (Bryan, 1968; White and Rainbow, 1982; Rainbow, 1990) might have occurred.

Cd uptake was also reported to exhibit a direct relationship with temperature in *M. edulis* (Lannig *et al.*, 2006; Mickelson, 2008). It was observed in this study for Cd uptake, precisely at low salinity where the uptake was higher at high temperature, which was 4.23 µg.g-¹.day-¹ that is almost double that at low temperature, which was 2.32 µg.g-¹.day-¹ (Fig.1). The same trend was reported for fiddler crab by O'Hara (1973a,b) and for *M. edulis* by Phillips (1976a) in which an inverse relationship existed between salinity and Cd uptake and this is clear at high temperature as observed in this study (Fig.1).

According to Table 4, Cd uptake was inversely related to salinity at high temperature and the difference between Cd uptake on day 14 at both salinity regimes at 6°C was 10.64 µg.g-¹, which might be related to periods of valve closure at low salinity (Gilles, 1972; Coleman, 1973), which is a stressful condition along with low temperature. Also, this could be due to elimination (e.g. Mubiana and Blust, 2007). Another observation at low salinity and low temperature was that the very small increase in uptake from day 11 to day 14 (0.40 µg.g-¹) (Fig.1). This might be an indication of an elimination period as observed by other authors, which have also observed for Zn as described earlier. Such elimination periods for Cd were also observed in other molluscs such as the green-lipid mussel *Perna viridis* (Yap *et al.*, 2003), the Asian periwinkle *Littorina brevicula* (Han *et al.*, 2003) and the Asiatic clam *Corbicula fluminea* (Qiu *et al.*, 2005). As for the high salinity and high temperature, the rate of uptake was 2.45 µg.g-¹.day-¹, which is only slightly lower than that of low temperature that was 2.51 µg.g-¹.day-¹ (Fig.1). This very slight difference at high salinity and high temperature could be related to the condition of mussels brought from the field or valve closure due to aerial exposure (Coleman, 1973).

There was a slight interaction between temperature and salinity (as explained in the results) for Cd uptake ($F_{1,64}$; $p=0.040$), which was very obvious at low salinity and high temperature.

It has been reported that Cd uptake by *M. edulis* is indirectly proportional to salinity (Phillips, 1976a; Jackim *et al.*, 1977) as in other molluscs such as *Mya arenaria, Mulinia lateralis* and *Nucula proxima* (Jackim *et al.*, 1977) and *Perna viridis* (Yap *et al.*, 2003). Furthermore, this phenomenon was also observed in crustaceans such as the fiddler crab *Uca pugilator* (O'Hara, 1973a,b) and the shore crab *Carcinus maenas* (Wright, 1977a,b; Bjerregaard *et al.*, 2005) under laboratory conditions. It has also been reported that in mussels the net uptake of Cd was unaffected by low temperatures at high salinities but was decreased at low salinities by low temperatures (Phillips, 1976a) as in this study, where Cd at low salinity and low temperature had the lowest rate of uptake (2.34 µg.g-¹.day-¹) and the lowest R-Sq (88.33%) value but the rate of uptake did not vary much from the rates of uptake at high salinity under both temperature regimes (Fig.1). Also, the increase in Cd uptake at higher temperature was less pronounced at high salinity than at low salinity (O'Hara, 1973a,b). This agrees with the results obtained during the present study (Fig.1) on *M. edulis* through the interaction between salinity and temperature ($F_{1,64}$; $p=0.040$) and the highest rate of Cd uptake (4.23 µg.g-¹.day-¹) at 20 PSU and 12°C.

Table 4. Cd and Zn concentrations in *Mytilus edulis* reported by Phillips (1976a) and this study in different conditions. All concentrations are in µg.g-¹. Values are means ± SD. The exposure period was 14-day in both studies. The overall mean ± SD for both Cd and Zn of control mussels at the different conditions is shown for both studies.

Cd	Zn	Condition	Control		Reference
1.22±0.57	45.8+26.8	18ºC, 35 PSU	[Zn]	23.73 ± 8.025	Phillips
62±2.04	51.9±24.4	18ºC, 15 PSU	[Cd]	0.50 ± 0.11	(1976a)
1.42±0.41	51.4±20.1	10ºC, 35 PSU			
2.25±0.66	41.4±12.7	10ºC, 15 PSU			
40.38±5.47	581.6±111.13	12ºC, 35 PSU	[Zn]	279 ± 57.49	This
56.94±11.06	462.4±69.86	12ºC, 20 PSU	[Cd]	0.84 ± 0.12	study
39.48±17.24	534.4±58.09	6ºC, 35 PSU			
28.84±7.3	420.8±22.43	6ºC, 20 PSU			

Mussels collected for this experiment from Lunderston Bay had a very high Zn concentration as presented in Figure 2 for the control values and the ranges for Cd and Zn for control mussels in this study were 0.014-1.4 and 130-536 µg.g-¹, respectively. Other data reported for control mussels in Clyde Estuary from March, 1980 to March, 1984 for the concentrations of Cd and Zn were 1.2-4.7 and 86-267 µg.g-¹, respectively (CRPB, 1985). Leung *et al*. (2001) have reported that the mean Cd and Zn concentration in mussels collected from Gourock (close to Lunderston Bay) were 0.57 ± 1.27 and 80.03 ± 24.69 µg.g-¹, respectively but Mickelson (unpublished data) has reported the following mean values 0.1-0.9 µg Cd.g-¹ and 53-96 µg Zn.g-¹ for the Clyde Sea Area but the mean concentration of Cd and Zn in Lunderston Bay during a study from October, 2007 to March, 2008 was 0.418 ± 0.396 and 90.52 ± 37.92 µg.g-¹, respectively.

As for the survival of mussels exposed to Cd and Zn during the study, at high salinity, the survival was 100% when exposed to both trace metals under both high and low temperature regimes (Table 3). While at low salinity, Cd survival was 64% at both temperatures but the survival when exposed to Zn at high temperature was 92% and at low temperature it was 60% and mortalities for both trace metals were observed only on day 14 of the experiment (Table 3). This could indicate that low salinity by itself was a stressful condition for mussels exposed to Cd and Zn. Mortalities for control mussels were only encountered at low salinity, in which the survival was 68% at 12°C temperature but it was 44% at 6°C (Table 3), which might indicate that both low temperature and low salinity had a dual effect on the survival. Nelson *et al.* (1988) also encountered some mortalities in their control molluscs but it must be considered that in that study the salinity used by Nelson *et al.* (1988) was 26±2 PSU with 3.5-13.8°C and most of the molluscs used were either juveniles or young-of-the-year. In contrast, the adult blue mussels controls had 94.4% survival over 126 days (Nelson *et al.*, 1988).

Conclusion:

In conclusion, there was an interaction between temperature and salinity on the cadmium uptake by *M. edulis* at which the rate of uptake of Cd was at its maximum at low salinity and higher temperature while temperature had almost no effect on Cd uptake at high salinity. These observations are similar to those of O'Hara (1973a,b) and Phillips (1976a). Cd uptake was almost linear with time at both salinity regimes at high and low temperature, which has also been reported in previous studies (e.g. Jackim *et al*., 1977; Everaarts, 1990; Tedengren *et al*., 1999; Vercauteren, 1999; Moolman *et al*., 2007; Widmeyer and Bendell-Young, 2007).

As for Zn, there was no interaction between temperature and salinity and the rate of uptake was significantly controlled by salinity while the effect of temperature was marginally significant as also observed by Phillips (1977b). Such observations allowed the rejection of the null hypothesis that salinity and temperature would have no affect on the rate of metal uptake.

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تأثير الملوحة ودرجة الحرارة على معدل أمتصاص المحار الأزرق الشائع *Mytilus edulis* مع بعض الملاحظات الخاصة حول بقائهم أحياء

محمد علي[1] و أ. تايلور[2]

[1]قسم الاستزراع البحري والأسماك، معهد الكويت للأبحاث العلمية، الكويت، [2]كلية العلوم الطبية وعلوم الحياة، جامعة جلاسكو، المملكة المتحدة

المستخلص يهدف هذا البحث لدراسة تأثير درجة الحرارة والملوحة على معدّل امتصاص المحار الأزرق (*Mytilus edulis*) لعنصري الخارصين (Zn) والكادميوم (Cd) في المختبر حيث أن تعرض المحار لهذين العنصرين بتركيز 2 ppm جاء بزيادة مضطردة تقريبا خلال 14 يوما (فترة التجربة). شرح المحار وأخذ نسيجه الناعم وتجميده، ومن ثم فك التجميد وهضمه في الحمض قبل قياس مستوى التلوث بجهاز تحليل الأطياف الضوئية (spectrophotometer). معدل امتصاص الخارصين بدا متقطعا بسبب فترات الإلغاء تحت الملوحة العالية بينما معدل امتصاص الكادميوم كان في قمته عند درجة الحرارة العالية والملوحة المنخفضة بينما لم يكن هناك تأثير قوي وواضح للحرارة في معدل امتصاص الكادميوم تحت الملوحة العالية. كما نشير إلى أن الملوحة أثرت بقوة في معدّل امتصاص الخارصين حيث كان الأعلى تحت الملوحة القليلة. أما فيما يتعلق بالوفيات فلم يلاحظ ذلك إلا تحت الملوحة المنخفضة للمحار المتعرض للعنصرين سالفة الذكر والمحار غير المعرض لأي عنصر (mussles control).